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10/574,530	07/21/2006	Keiichi Fukuda	58777.000019	9902

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EXAMINER	
BERTOGLIO, VALARIE E	
ART UNIT	**PAPER NUMBER**
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/574,530	FUKUDA ET AL.	
	Examiner	Art Unit	
	Valarie Bertoglio	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on *11/07/2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-18* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-18* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on *N/A* is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *07/08,04/07,04/06*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's election without traverse of Group I, claims 1-18 as they relate to use of pluripotent stem cells in the reply filed on 11/04/2008 is acknowledged. No claims are withdrawn, however, the claims read on non-elected subject matter relating to use of multipotent adult stem cells. Claims will be examined to the extent that they read on the elected invention.

Claim Objections

Claims 1,3-12 and 14-18 are objected to because of the following informalities: The claims read on nonelected subject matter and should be amended such that they encompass only use of pluripotent stem cells. Appropriate correction is required.

Claim Rejections - 35 USC § 112-1st paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-13 and 15-18 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of differentiating mouse pluripotent stem cells into cardiomyocytes comprising culturing the pluripotent stem cells in the presence of a BMP antagonist wherein the antagonist is present in the culture media for 3 days prior to the differentiation stage and for no more than 3 days following the induction of differentiation, does not reasonably provide enablement for the claimed method 1) using BMP antagonists at any other time of culture, 2) use of non-mouse pluripotent cells, 3) use of BMP signaling inhibitors other than BMP antagonists or 4) administering the BMP antagonist through any means other than addition of protein to the culture medium. The

specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Enablement is considered in view of the Wands factors (MPEP 2164.01(a)). The court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (*Wands*, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (*Wands*, 8 USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case are discussed below.

The claims are drawn to a method for inducing differentiation of pluripotent stem cells (i.e. ES cells, EC cells or EG cells) into cardiomyocytes by culture in the presence of inhibitors of BMP signaling. The claims broadly encompass culture with BMP antagonists that directly prevent the binding of extracellular BMPs with their signaling receptors as well as other inhibitors that act at various points in the intracellular BMP signaling cascade. Claims also encompass any species of stem cell including all mammals and non-mammals. Dependent claims limit the BMP inhibitor and others limit the required culture period in the presence of BMP inhibitors. The claims are broad in encompassing non-elected

multipotent stem cells and are not examined with regard to this encompassed, patentably distinct subject matter.

The specification has taught culture of *mouse* pluripotent ES cell in the presence of BMP antagonists noggin and chordin as well as other BMP antagonists known in the art. In Example 1, 3 days before the start of floating culture to allow the differentiation of the ES cells, the cells were treated with 500ng/ml of mouse noggin protein in the presence of LIF, which prevents differentiation of mouse ES cells. Following, floating culture in the presence of 500 ng/mL of noggin for 3 days was performed to initiate differentiation into EBs. Similarly, the hanging drop method in the presence of 500 ng/ml noggin was also performed to form EBs (paragraph [0070]). These methods found that there was a significant increase in the number of beating (cardiomyocyte) EBs when treated with noggin as compared to EBs not treated with noggin (paragraph [0071]). In the hanging drop culture, it appeared there were also more beating cardiomyocytes in the EB. The specification discusses that addition of BMP-2 under the same conditions as noggin did not have effects equivalent to or greater than noggin (paragraph [0074]). Variations in the concentration of Noggin used in this method revealed optimal concentrations of 50-150 ng/ml (paragraph [0074]).

Example 3 compares differences in treatment time and period and teaches that treatment with Noggin at both the predifferentiation and differentiation inducing stage are necessary to obtain a positive effect on directed cardiomyocyte differentiation (paragraph [0085]). Culture with LIF prior to differentiation was also required. Importantly, presence of Noggin in the culture medium beyond day 5 inhibited cardiomyocyte differentiation (paragraph 0087]). Example 4 teaches that including 5 mg/ml BMP-2 in the culture of Noggin treated ES cells during the predifferentiation period and 3 days in differentiation conditions negated the effects of Noggin in inducing cardiomyocyte differentiation. Example 5 found similar results when using Noggin in the culture of ES cells on a feeder layer (paragraph

[0092]). Examples 6 and 7 followed the same method with the BMP antagonists Chordin, Follistatin, DNA, Caronte, and Gremlin and arrived at similar results.

The art at the time of filing reported seemingly contradictory findings in that BMPs are effective in directing differentiation of ES cells into cardiomyocytes while BMP antagonists such as Noggin, direct differentiation along neural fate paths. Monzen et al (2001, IDS) taught that constitutive overexpression of the BMP inhibitor Noggin in P19CL6 pluripotent cells does not allow for differentiation into cardiomyocytes even in the presence of the if the potent cardiomyocyte differentiation factor DMSO, suggesting that BMPs are essential for cardiomyocyte differentiation. Circumventing the Noggin inhibition using downstream signaling molecules (SMADs) reversed the inhibitory effect of Noggin, resulting in DMSO induction of cardiomyocyte differentiation in Noggin expressing cells. Monzen differs from the teachings in the specification in that Noggin is present constitutively while the specification teaches that it is essential to remove Noggin from the differentiation culture prior to day 5 (paragraph [0087]) and only teaches effective methodology when it is removed at day 3. However, with exception of claims 16 and 18, Noggin is not required to be removed after day 3. In the case of claim 16, Noggin is not required in the predifferentiation medium which is required to obtain the effect of increasing cardiomyocyte differentiation to 80% of EBs as occurs with the conditions of claim 18.

Bin et al discuss that the signals required for cardiomyocyte differentiation are complex and only partly understood [2006, **Cell Biology International**, 30:769-776]. Bin induced cardiac differentiation by removing LIF from the culture medium and after 2 days, cell aggregates were implanted onto coverslips in the presence of BMP-2. Cardiac induction was observed by day 3 post-implantation, or day 5 following removal of LIF. Rajasingh et al concurred that the stimuli and signaling pathways that define cardiomyocyte differentiation had yet to be defined as of 2007 [2007, **Circ. Res.** 101:910-918]. Rajasingh examined the effects of varying dosages of LIF, BMP and BMP pathway inhibitors on ES cell differentiation into cardiomyocytes. LIF and BMP2 were found to synergistically enhance cardiomyocyte

differentiation. Two days of culture with BMP2 in the presence of LIF was sufficient to induce cardiomyocyte differentiation (see Figure 1). Accordingly, Yuasa et al report that it is the *transient* expression of Noggin that induces cardiomyocyte differentiation and that the inductive activity of Noggin is restricted to a period of 3 days before and 1 day after EB formation [2005, **Nature Biotechnology**, 23:607-611]. Yuasa et al. state that BMP signaling is essential for at least two steps in the cardiomyocyte induction process: mesodermal induction and cardiomyocyte differentiation. However, Yuasa finds that between these two steps a transient block of intrinsic BMP signaling may be the most important step. Thus, the claims fall subject to the precise timing requirements of the presence of noggin in ES cell differentiation as exemplified by the art.

The claims also encompass use of any species of ES cell in generating cardiomyocytes. Ruhnke taught the unpredictability of isolation and directed differentiation of pluripotent ES cells in various species [2003, **Stem Cells**, 24:428-436]. Ruhnke taught the isolation of ES-like cells from rats, however, found that these cells behaved differently in culture (page 434). Likewise, Keller discusses that human ES cell growth is less understood than that of mouse ES cell growth [2005, **Genes and Development,** 19:1129-1155]. Wang et al [2005, **BBRC**, 330:934-942] taught that human pluripotent ES cells differ from mouse ES cells and require a mouse embryonic fibroblast feeder layer to provide factors that prevent differentiation. LIF is not sufficient to prevent differentiation in the absence of a feeder layer. Wang et al found that human ES cells are effectively prevented from differentiating in the presence of 500 ng/ml Noggin, a role not established in the mouse ES cells treated with noggin as discussed in the specification. The art, thereby establishes an unpredictability as to the applicability of the culture conditions between species of pluripotent stem cells to obtain desired differentiation results. Thus, the specification fails to demonstrate that the culture conditions that direct differentiation of mouse ES cells to cardiomyocytes

would have the same effects on pluripotent cells of other species. Hence, it would require further experimentation to determine how to differentiate non-mouse ES cells into cardiomyocytes.

In light of the complexity and sensitivity of the cardiomyocyte differentiation and the important and specifically timed effects of two opposing signaling mechanisms, it would also be unpredictable how to apply the teachings of the specification to other routes of administering the BMP antagonists. The specification has taught addition of extracellular BMP antagonists to the culture medium. The specification does not provide any guidance relating to other modes of introducing the BMP antagonists such as via recombinant gene expression. Due to the precise nature of the timing of these opposing signals, it would require undue experimentation for one of skill in the art to determine how to temporally control BMP protein presence in the culture medium through any other means. Furthermore, the claims encompass use of BMP signaling inhibitors that act intracellularly. The specification fails to provide any guidance regarding how to introduce these factors into the cell at the precise and necessary time points. As well, due to the use of downstream signaling molecules in multiple and disparate pathways, it would not be predictable what other signaling pathways would be affected by such inhibitors and whether or not those effects would alter cardiomyocyte differentiation.

Thus, the art has established that at the time of filing there were several unpredictabilities surrounding the claimed methods. First, the timing of various signaling cascades is very complex and the presence or absence of BMP signaling must occur appropriately at characteristic stages of differentiation. Second, these windows of timing as well as the effect of different factors and signaling cascades vary between species of ES cells. Thus, the specification is only enabling for use of BMP antagonists in the culture medium to direct differentiation of pluripotent stem cells into cardiomyocytes under the precise conditions discussed in the specification. Because these conditions cannot be predictably applied to other species, the specification fails to enable use of pluripotent stem cells from species of animals other than

mouse. It would require undue experimentation for one of skill in the art to determine how to use the culture conditions broadly claimed to direct cardiomyocyte differentiation from pluripotent cells and, as well, would require undue experimentation to determine a time course of BMP-inhibitor treatment of pluripotent cells other than mouse cells to arrive at cardiomyocytes as claimed.

Claim Rejections - 35 USC § 112-2nd paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim1-18 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim Claims 36-40 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is incomplete as written. The preamble of the claim is directed to a method for inducing differentiation of cardiomyocytes from stem cells. However, the claim is incomplete because the method steps do not relate back to the preamble in a positive process. The claims fail to require that a cardiomyocyte be made. Claims 2-18 depend from claim 1.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-18 are rejected under 35 U.S.C. 102(b) as being anticipated by Monzen (1999, IDS).

Claims 1-13 and 15-18 are drawn broadly to a method of culturing stem cells in the presence of a BMP inhibitor to induce differentiation. Claim 14 is drawn to a cardiomyocyte made by the method of claim 1 and is a product by process claim where the process by which the product is obtained is given no patentable weight as the cardiomyocyte fails to differ from any other cardiomyocyte.

As such, the limitations of the claimed cardiomyocyte are met by any cardiomyocyte in the prior art. Patentability of a product-by-process claim is determined by the novelty and nonobviousness of the claimed product itself without consideration of the process for making it which is recited in the claims. *In re* Thorpe, 227 USPQ 964 (Fed. Cir. 1985).

Monzen taught culture of mouse ES cells in the presence of noggin and the cells developed into cardiomyocytes (page 7098, col. 2, paragraph 2). Monzen also taught a cardiomyocyte (see page 7098, col. 2).

Thus, Monzen meets the limitations of claims 1-18.

Claim 14 rejected under 35 U.S.C. 102(b) as being anticipated by Zhang [2001, **Am J Physiol Heart Circ Physiol**, 280:H1782-1792].

Claim 14 is not limited to an isolated cardiomyuocyte and therefore reads on a cardiomyocyte, in vivo. Furthermore, claim 14 is a product by process claim where the process by which the product is obtained is given no patentable weight as the cardiomyocyte fails to differ from any other cardiomyocyte.

As such, the limitations of the claimed cardiomyocyte are met by any cardiomyocyte in the prior art. Patentability of a product-by-process claim is determined by the novelty and nonobviousness of the claimed product itself without consideration of the process for making it which is recited in the claims. *In re* Thorpe, 227 USPQ 964 (Fed. Cir. 1985).

Zhang taught a heart comprising cardiomyocytes (see Figure 5, for example).

Thus, Zhang meets the limitations of claim 14.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Valarie Bertoglio whose telephone number is (571) 272-0725. The examiner can normally be reached on Mon-Thurs 5:30-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272-4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Valarie Bertoglio/
Primary Examiner
Art Unit 1632